workers have been careful to cover the decapsulated kidney with omentum so as to develop a collateral renal circulation(14). In the present experiments, after decapsulation, the kidneys remained in their normal anatomical relationships. At autopsy the decapsulated kidneys were surrounded with the usual perirenal adipose tissue and there was no evidence of any collateral circulation. However, the decapsulated kidneys did present rather bizarre, lobulated forms and were invariably somewhat larger than the normal kidneys of corresponding animals, both choline deficient and non-choline deficient.

Whatever may be the role of the capsule in this situation, it cannot be the sole factor involved. As shown by the effects of ACTH, even after decapsulation, there remains considerable functional impairment. It has been found in this laboratory that ACTH, in this same dosage, will restore the hypertension of rats from which ¾ of their renal tissue has been removed and in whom hypertension has been prevented by feeding a low protein diet(8) and will also produce hypertension in bilaterally nephrectomized rats while they survive(15), yet is without effect on unilaterally nephrectomized rats. Thus, the renal decapsulated, normotensive post-choline deficient rats of the present study may be considered to have, in this sense, renal function comparable to that of an animal with somewhat less than one intact kidney. There may be an analogy between such rats and humans who develop hypertension under stress or 'tension' yet appear to have normal renal function by the usual criteria.

Summary. Bilateral renal decapsulation, performed one week after an acute episode of 'hemorrhagic kidneys' in choline deficient rats, prevents the development of hypertension in such animals and restores blood pressure to normal when the operation is performed in rats in which such hypertension is already established. Administration of ACTH to such renal decapsulated rats restores their hypertension but is essentially without effect on the pressures of normal rats, hypertensive rats, or renal decapsulated rats which never were choline deficient.

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A Microbiological Assay Method for Biotin. (18484)

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Microbiological methods for the determination of biotin have been employed in many laboratories using several microorganisms (*Lactobacillus casei, L. arabinosus, Clostridium butylicum, Leuconostoc mesenteroides, Rhizobium trifolii*)(1,2). Although 2 molds (*Neurospora crassa* wild and cholineless, and *Nematospora (Ashbyia) gossypii*) have been used as test organisms for biotin(3-5) they have not yet been generally used.

In the present paper a new method is described based on the biotin requirement of the filamentous fungus *Allescheria boydii*. This observation was first reported by Cury (6) and a more detailed study on vitamin nutrition related to biotin was published by Villela and Cury(7). It has been shown that *A. boydii* is a specific organism for biotin and that the growth responses are proportional to the amount of this vitamin in the medium.

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TABLE I. Basal Medium.

Dextrose (anhydrous)	30.0 g
Asparagine (biotin-free)	2.0 "
$MgSO_4 \cdot 7H_2O$	0.5 "
KH_2PO_4	1.5 "
Trace elements sol.*	1.0 ml
Distilled water to	1000 "
pH adjusted to 5 with 2 N NaOH	

* The stock solutions of trace elements consist of: 28.2 mg of H_3BO_3, 81.6 mg of $CuSO_4 \cdot 5H_2O$, 40.55 mg of $MnSO_4 \cdot 4H_2O$, 49.7 mg of $FeSO_4 \cdot 7H_2O$, 18.36 mg of $(NH_4)_6Mo_7O_{24} \cdot 4H_2O$, and 395.6 mg of $ZnSO_4 \cdot 7H_2O$ in 1000 ml of distilled water.

Therefore, we thought that it would be useful to work out an assay method for biotin.

Experimental. Organism. The test organism used is the strain of *Allescheria boydii* originally isolated by Boyd and Crutchfield from a mycetoma of yellow-white grains (strain N° 1699 of our culture collection). However, it was not possible to demonstrate experimentally any pathogenicity for animals with this organism. The other 4 strains investigated are unsuitable for the test since they grow without biotin. We believe that our strain N° 1699 is a natural mutant which has lost its capacity for synthesizing biotin. Stock cultures of *A. boydii* are maintained at room temperature by monthly transfers in a medium consisting of 3% dextrose, 1% peptone (Difco), 1% malt extract (Difco), and 1.5% agar.

Basal medium. The composition of the basal medium is shown in Table I.

Procedure. Assays are carried out in 50 ml Pyrex Erlenmeyer flasks cleaned with sulfuric acid-dichromate solution, thoroughly washed with tap water and rinsed with distilled water. The medium is made in double concentration and 5 ml distributed to each flask. After the convenient volume (1 to 5 ml) of the biotin standard solution or the extract to be tested are added the volume is completed to 10 ml with distilled water. The flasks are plugged with white cotton and autoclaved for 15 minutes at 120°C. In order to establish the curve for assay the stock biotin solution (50 μg per ml) is diluted to suitable concentrations. Aliquots of these diluted solutions are added to the flasks in duplicate in order to obtain 1 to 100 mμg of biotin per flask. A typical curve is shown in Fig. 1. Samples for assay are added in other flasks in volumes estimated to contain between 1 to 100 mμg of biotin. Volumes up to 5 ml may be used. The spore suspension for the inoculum is prepared by adding 10 ml of the basal medium to a culture and scraping the surface of the culture slightly with a platinum loop. A drop of the suspension is inoculated directly into the medium in each flask. The flasks are rotated to obtain a uniform mixture and incubated at room temperature for 8 days. They are then autoclaved for 15 minutes at 115°C. Then the pads are removed, washed thoroughly with distilled water, pressed out on filter paper, rolled in pellets, and dried at 110°C for 2 hours. After being cooled in a desiccator, they are weighed on an analytical balance. A reference curve with pure biotin should be established for each day's run. Controls (basal medium alone) are run parallel in all assays. The biotin content of the test substance may be determined by comparing the growth response of the unknown with that obtained with pure biotin. The average values encountered at 2 or more levels is used. Values taken below 1 and above 100 mμg should not be considered.

Reliability of method. The reliability of

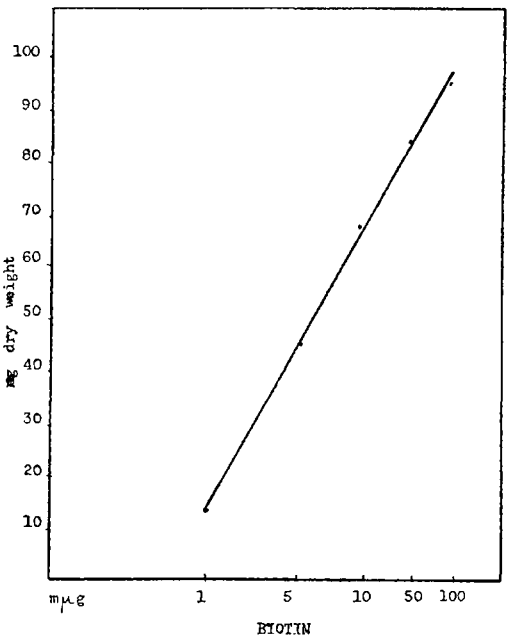

FIG. 1.
Growth response curve of *A. boydii* to biotin.

TABLE II. Reproducibility of Results with Known Amounts of Biotin.

Biotin, μg/10 ml	Dry wt (mg)										Mean and σ
.001	12.8	12.3	12.8	13.4	13.0	13.4	13.0	12.8	12.4	13.0	12.9 ± 0.36
.01	64.8	69.0	63.8	69.8	63.0	69.8	65.9	69.4	69.9	65.2	67.0 ± 2.88
.1	83.9	86.8	81.0	83.0	89.6	85.2	84.3	83.0	84.8	84.6	84.6 ± 0.74
.0	.0	.0	.0	—	—	—	—	—	—	—	

$$\sigma = \text{Standard deviation} = \sqrt{\frac{\Sigma (x-m)^2}{n-1}}.$$

the method is indicated by good reproducibility of the determinations with pure biotin. As shown in Table II the reproducibility of the data are acceptable.

Preparation of samples for assay. For complete extraction of biotin from foods and tissues and liberation of biotin from the bound-biotin complexes the ground or blended samples are autoclaved in a convenient volume of 6 N HCl(1). After cooling the extracts are adjusted to pH 5.0 with N sodium hydroxide solution.

Specificity. The specificity of biotin for *A. boydii* has been studied(7). It has been shown that the biotin analogs (O-heterobiotin and dl-desthiobiotin) are able to replace biotin partially in the growth of *A. boydii*. When associated with biotin these analogs do not show an additive response. Cumulative effects are, however, observed only with amounts of 50 mμg or more of these analogs. Since these synthetic analogs are not found appreciably in natural products they will not interfere in the assays. It was also observed that nicotinic acid has some stimulative effect when associated with biotin. On the other hand, pyridoxin has a depressive effect on growth. This depressive action is neutralized by nicotinic acid. It is possible to avoid the interference of these vitamins by the use of selective adsorbents. Cysteine, glutathione, pimelic acid, aspartic acid, oleic acid and "Tween 80" are not effective in promoting the growth and do not interfere when associated with biotin. Biocytin, a naturally occurring complex of biotin recently isolated from yeast by Wright and coworkers(8), is effective in promoting the growth of *A. boydii* (9) as well as of *Saccharomyces cerevisiae* (10) although inactive for *Lactobacillus arabinosus*(8).

TABLE III. Assay Values and Recovery Experiments.

Material assayed	Biotin added per g sample, μg	Biotin found per g sample, μg	Recovery of added biotin, %
Rat liver	0	.75	—
	.17	.93	101
Yeast extr.	0	.67	—
	.33	1.06	106
Casein (vit. free)	0	0	—
	.25	.25	100

Biotin assays and recoveries. Table III contains the assay values and recoveries of added biotin for 3 biological materials (rat liver, yeast extract, and purified casein). There are good recoveries of the added vitamin and the assay values given agree favorably with those reported in the literature.

Summary. A microbiological method for the quantitative determination of biotin is described. *Allescheria boydii* 1699 is used as test organism. The reliability of the method is indicated by the reproducibility of assays and good recoveries of the biotin added to various samples. The method is sensitive, the assay range being 1 to 100 mμg of biotin.

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